Cambridge University Underwater Exploration Group

MALTA EXPEDITION

1965

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Cambridge Malta Expedition 1965.

During the period July 6th till August 17th six undergraduate divers from Cambridge carried out a marine zoological project off the coast of Malta. It was hoped that we would find nocturnal behaviour in at least some of the native marine animals, and we were fortunate in finding, during the first two weeks, a number that did show just this phenomenon.

After a few night dives at Wied iz Zurrieq it became clear that a species of Holothurian present in large numbers during the hours of darkness was rarely if ever seen during the day. Also the Ophiuroids were more active at night, while a number of common fish present during the day were not seen at night.

Encouraged by this confirmation of our observations in Marseilles last year, we proceeded to analyse this phěnomenon in greater detail. After examination of a number of possible working sites, we decided on Wied iz Zurrieq for all our work. In this way we hoped to benefit from an intimate knowledge of the area in which we were working, and thus avoid the danger of wasting time on exploratory diving.

We soon found that we could best use our small team by dividing the work into three sections, running the projects cocurrently, and distributing the divers as necessary each day. Accordingly the following report is divided into three sections dealing with Holothurians, Echinoids, and Fish in that order.

Observations made on the Diurnal Activity and some other Aspects of the Behaviour and Distribution of Regular Echinoids.

The sharp-spined regular echinoids Paracentrotus lividus Martensen and Arbacia lixula Loven are amongst the most abundant sub-littoral invertebrates in Malta. Two other echinoid species, Sphaerechinus granularis Agassiz, and Centrostephanus longispinus Peters are occasionally to be found on the cliff and loose-rock substratum. Because of their abundance the work was conducted on Paracentrotus and Arbacia, with a brief look at Sphaerechinus. Paracentrotus and Arbacia could be differentiated between easily in the field after only very little practice.

Diurnal Activity.

Movement, as a criterion of activity, was followed over a 24 hour period in a series of labelled echinoids of both species.

Method: The movements of individual echinoids were measured relative to a set of fixed points on the substratum using a simple Triangulation method. The fixed points consisted of neoprene or cork floats attached by twine to the substratum. The spatial relationships of these to each other were determined, and the position of each echinoid measured relative to any three. The results were then scaled down (1/20) enabling a map of the movement to be plotted. From this the graphs of percentage movement were produced.

The problem of labelling echinoids underwater, disturbing them as little as possible, proved to be a difficult one. Barbed points (straight fish-hooks) with numbered plastic tags were used first. It was hoped originally to insert the point in the flexible periproctal membrane around the anus, but, owing to the small size of the urchins, the tendency was to insert it into the anus, with ejection of the point and rapid fatality. It was then found that the points could be tapped gently into the test. The initial reaction of the urchins to the point was rather vigorous, with considerably increased movement. In one Arbacia in particularly favourable circumstances, it was observed that the movement was directly away from the position and direction of insertion, and the range of movement was 25 cms in the first five minutes, compared with an estimated average daily movement in echinoids of these species of 2 M.

Experiment 1: Four days after labelling eight echinoids, one Arbacia and one Paracentrotus were followed over 24 hours, observations being made every three hours. The results obtained did not show any periodicity for these two individuals. Within a week to a fortnight from this 24 hour watch, however, all labelled echinoids were dead. The success of the

Oxford Expedition using stainless steel points, and the rusty appearance of the intra-echinoid part of our points lead us to suggest that the tin plating of ours tended to dissolve and poison the animal. Clearly, normal activity was being masked by the animals' response to this chronic noxious stimulus.

Another labelling method had to be devised. C.C.Hemmings suggested sliding small pieces of plastic sleeve insulation from electric wire over individual spines. This proved possible, and the initial reaction consisted of a brief local spine response, without, to the best of our knowledge, any increase in locomotor activity. The echinoid will shed the labelled spine within 36 - 48 hours. Thus to avoid losing track of our experimental animals a system of multiple tagging (two or three per animal) and a regular transfer of the labels to other spines every day was used.

Experiment 2: A 24 hour watch was carried out on an experimental group of six echinoids, four Paracentrotus, and two Arbacia. Because of the increased numbers, observations were made every four hours. The results were plotted as before. Five individuals followed a similar pattern, and the result for this group is represented in Fig.1.

It can be seen that in three Paracentrotus and two Arbacia there is a marked increase in the distance travelled during the period of darkness compared with the daylight hours. On average approximately two thirds of the total movement occurred during the eight hours of darkness. If the distance travelled can be taken as a function of the activity of the animal it appears that there is a marked diurnal change in the activity of these five echinoids.

The one sea-urchin which did not show an increase in movement at darkness was a paracentrotus, especially selected because it rested threequarters buried in a close-fitting hole in the rock. Many observations have been made on the rock-burrowing activities of echinoids, and it was hoped that this individual would move from and return to its hole, i.e. that it would show some sort of homing activity. However, from the time it was labelled on the 3rd August until the last dive on the 15th August, observations at different times of the day and night, including the 24 hour watch, showed this individual to remain in its hole. No locomotory activity was ever seen to occur.

The direction of movement during any period is not predictable, and thus our measurements approach fairly closely, but do not exactly equal the true distance. As we are comparing percentage movement over different periods, these errors should largely cancel out.

Vertical Distribution of Paracentrotus and Arbacia.

Three metre-wide vertical transects were carried out from the base of the cliff (100 feet) to the surface to see whether there was any change in the species composition of the Paracentrotus/Arbacia population. A count was made of all echinoids within a half-metre band to either side of a line laid down the cliff face. The numbers were totalled every 10 feet of depth, and are presented in Fig. 2.

Transect C should be especially noted as this was taken up a vertical rock face from 100-0 feet. In transects A & B the numbers in the range 10-40 feet are grossly exaggerated because of an approximately 40° (to horizontal) shelf between these depths. The relationship between the peaks of the two populations, however, remains much the same, that of Arbacia being 10-20 feet above that of Paracentrotus.

As Arbacia is more frequent in the southern Mediterranean than in the north, one is tempted to think that this is a temperature-controlled distribution but it appears that the temperature difference between these two peaks of population is too small to be the decisive factor. It could be that algal distribution and/or light intensity are important.

Covering.

Many echinoids are seen to cover themselves with bits of weed or other extraneous material. It is claimed (Dubris 1913) that this is a protective measure against light. Observations made on this expedition, however, tend to disprove this theory.

1. Three rough counts at different depths (10, 30, & 80 feet) showed, firstly, that Paracentrotus tends to cover itself whilst Arbacia does not, and, secondly, that with increasing depth there is no significant change in the numbers of the population covered.

	Arbacia	P-centrotus	Arbacia	P-centrotus	Arbacia	P-centrotus
Covered	5	56	11	76	0	82
Uncovered	74	16	63	25	11	13
Depth	10 feet		30 feet		80 feet	

2. A daily check on the covering of the six 'sleeve-labelled' echinoids showed that both Arbacia, and one Paracentrotus never had any cover. For the other three the results are as follows :

Date of Observation.	A	B	C
3/8/65	+	+++	-
4/8/65	++	+	+

Date of Observation.	A	B	C
5/8/65	-	-	-
6/8/65	++	+	-
8/8/65	+	+	+

+ Denotes degree of covering   - No covering

As there was little, if any, variation of light intensity between any of these daily observations (all at the same depth, at midday, the weather being sunny), this variation in cover is surprising if it is dependent on light intensity.

3. On one dive, when heavy seas had caused an increase in floating detritus, it was noticed that many more echinoids than usual were carrying extraneous material.

From the above observations it appears that covering with extraneous material is not necessarily a response to high light intensity. It could be due to a local reflex on the part of the tube feet initiated by contact with any suitable surface. Material is actively held onto by the tube feet, not simply stuck over the spines.

Conclusions.

Both Arbacia lixula, and Paracentrotus lividus appear to exhibit a diurnal locomotor rhythm, being more active at night than during the day.

The peak of the Paracentrotus population occurs 10-20 feet below that of the Arbacia population. This distribution is probably determined by factors other than temperature.

Covering is not an active shading response, but is probably due to the tendency of the tube feet to cling onto any suitable surface.

Reference.

Dubois R. 1913. "Note sur l'action de la lumiere sur les echinoderms". C.R.9. Internation.Congr.Zool.

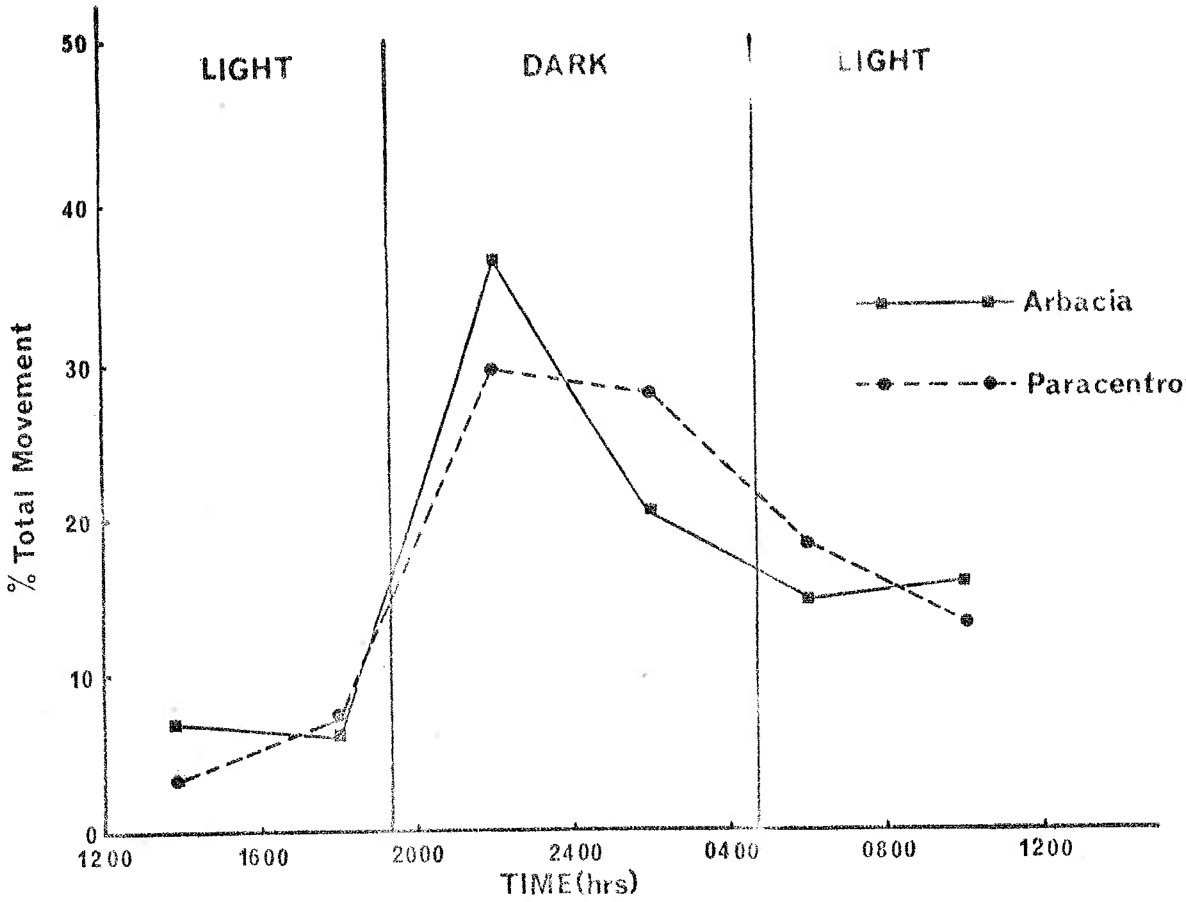


FIG. 1. % TOTAL MOVEMENTS DURING 24 Hr. WATCH 4-5th AUGUST 1965.
AVERAGE FOR PARACENTROTUS AND ARBACIA.

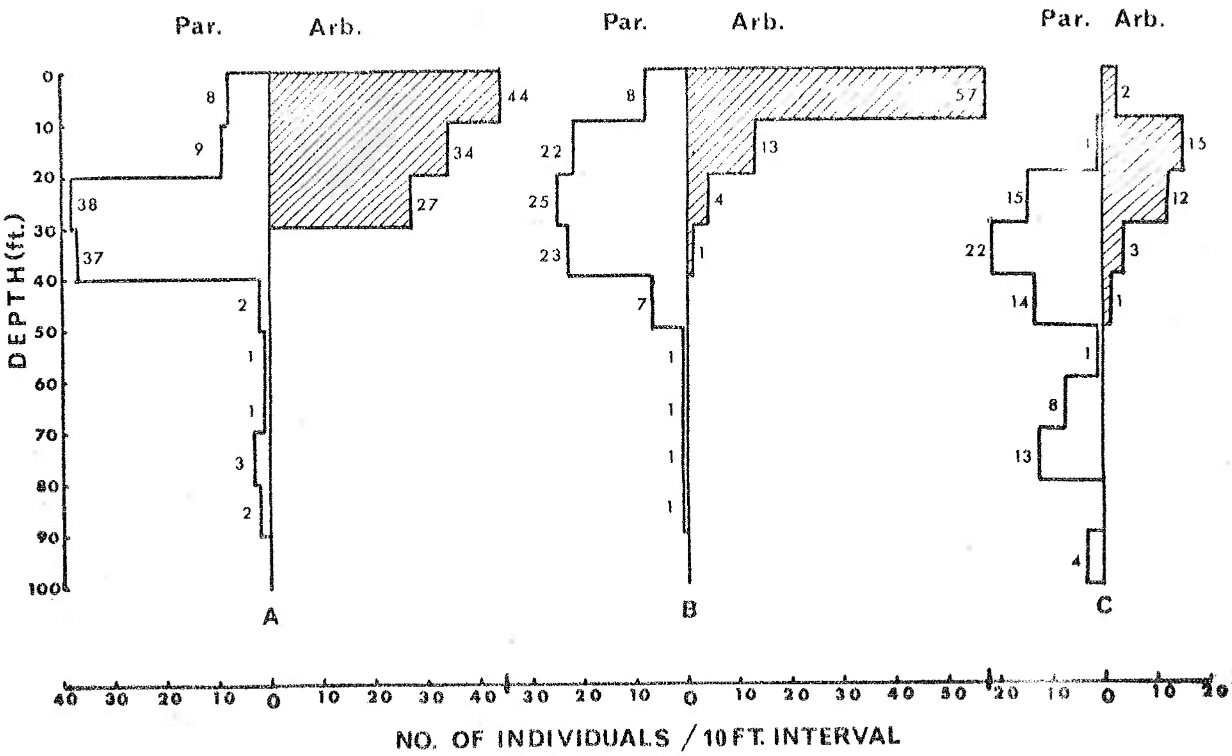


FIG. 2. COMPOSITION OF THE PARACENTROTUS (PAR.) / ARBACIA (ARB.) POPULATION
AS SHOWN IN 3 METRE-WIDE LINE TRANSECTS FROM 0-100 Ft.

Diurnal Activity in Holothuria.

On one of the first night dives we found a large rock on a sloping rock-debris bottom with a local population of about a dozen Holothurians, and we decided to concentrate our observations on an area four metres square around this rock. During the day there were no holothurians to be seen in the area, and from casual observations of the site made while doing a 24 hour watch on the Echinoids, it became apparent that these creatures are nocturnal. They seem to crawl out from holes in the substrate at dusk, browsing actively during the night, and going back into holes before dawn. To confirm this fact we watched the area from 1830-2000 hours, and were able to observe 14 animals emerge from holes amongst the rocks, and begin moving around in that time. This dusk observation was repeated several nights running with almost identical results.

Having confirmed the dusk emergence, the next obvious step was to attempt a continuous watch on the site during the hours of darkness which we did with the help of Hemmings, Lythgoe, et al. Thus we had ten divers and enough air to complete 12 one-hour dives if this proved necessary, although the period 1845 - 0500 hours proved sufficient for our purpose. The first diver went down at 1845, and plotted the positions of the animals, more or less as they appeared, on prepared formica boards every 15 minutes. He was relieved at 2000 hours by a second diver who continued to map the position of each animal at quarter-hour intervals. Continuity was achieved by the incoming diver receiving from his predecessor a formica board showing the last observation made. It was hoped in this way to get an idea of the movements of each individual, but in fact the identification proved most difficult and confusion about the identity of individual holothuria made continuous tracking impossible. Red torches were used for all observations as it had been found earlier that, while holothurians are very sensitive to white light, they are much less so to red.

Each individual was recorded on a formalised diagram of the site (Fig. 3) by a number and pattern designation, and the position estimated from metre tags along the peripheries and the central cross-line. Three types of Holothurians were distinguished for experimental purposes dependant on whether or not white or cream rings were present around the bases of the tubercles - Portholed, 'P', non-portholed, 'N', and an intermediate type in which only a small number of tubercles were ringed, 'p'. In general even this was not sufficient guide to the succeeding diver in identifying each animal, with certainty, especially when several were close together.

Despite the inability of divers to identify unmarked individuals it was possible to get a very clear idea of the numbers of individuals in the area at quarter hour intervals throughout the night. The results of this

10 hour continuous watch and several dusk watches are shown in Fig. 3.

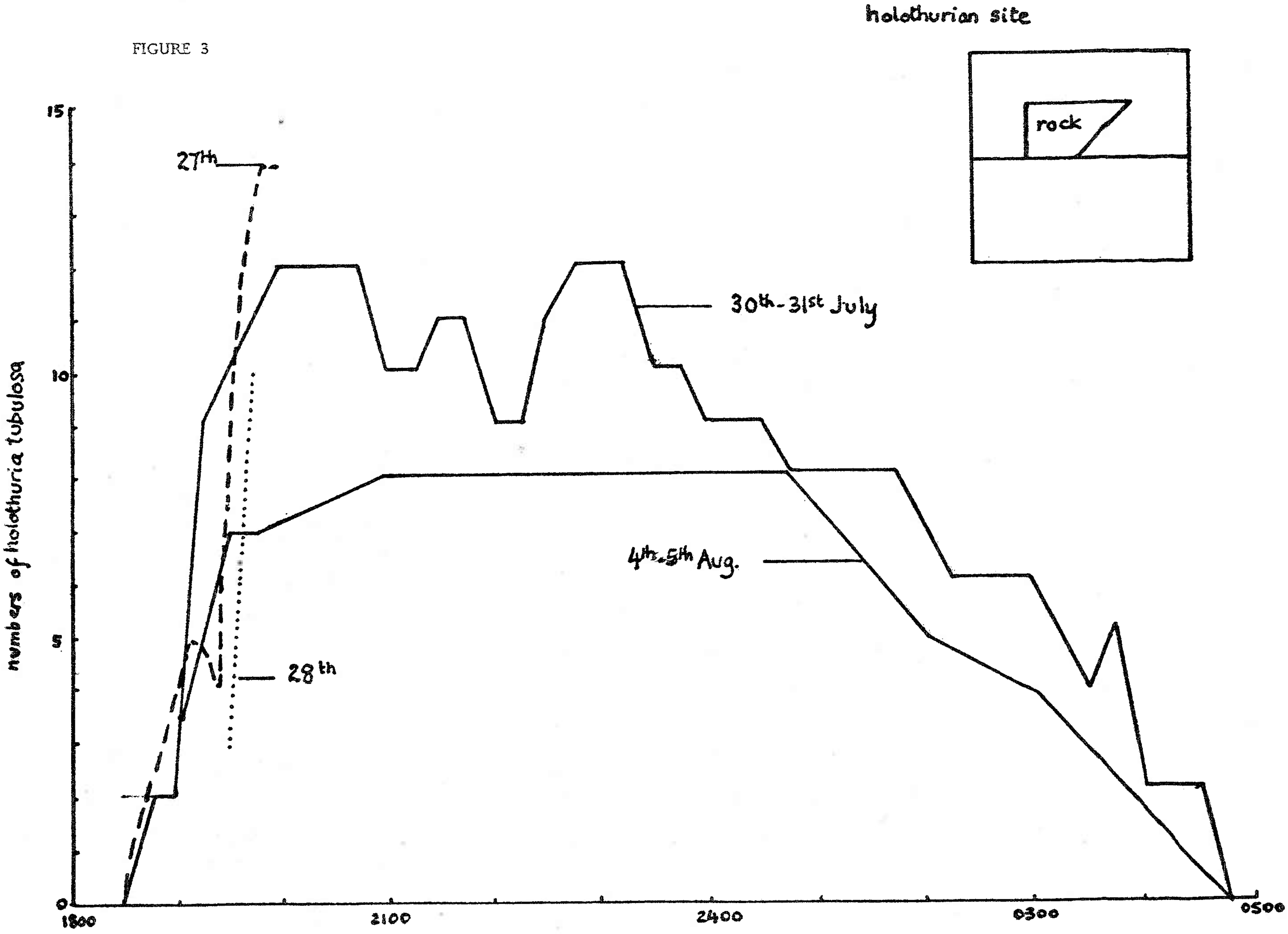
It will be seen that the curves in the period 1800-2000 hours fit very closely indeed showing the consistent nature of the dusk emergence. It will also be noticed that the principal curve (labelled 30-31st July) representing the 10 hour watch begins to tail off towards 0100 hours, and the number of animals present in the area falls off long before dawn. It is possible that we have forced off our population with fairly continuous exposure to red light. There is no reason, however, to suppose that the animals should go back into holes at the same time; they may go back beneath the substrate as soon as they have eaten their fill. The curve labelled 4 - 5th August represents observations made at more sporadic intervals throughout the night, avoiding the complication of continuous illumination, but does not disprove either theory.

Despite these problems we would suggest that these observations do show very clearly a nocturnal behaviour in Holothuria tubulosa (identified from 'Fauna der Adria'). These creatures do emerge from holes in the substrate within a comparatively short period around sunset (approx 1930 hours) and do move actively at night, browsing the diatom film on the surface of the rocks, and finally disappearing into holes sometime before dawn.

We did not find a satisfactory method of marking these animals, and it was almost impossible to map movements of individuals without marking them. Even so, what evidence we did glean from the 10 hour watch does not indicate a 'homing instinct' in these creatures. One or two animals of the same species were seen at other sites at greater depths during the day but never actively moving around and browsing on the surface as at night.

The dusk emergence was so closely linked to the dusk period 1800-2000 hours that it seemed likely that the phenomenon was controlled by either (1) a change in light intensity or (2) absolute light intensity. From this point the work could have been done more conveniently and accurately in aquaria under laboratory conditions. However, Dr. J. Lythgoe found a cave population of the same species of Holothurian, and suggested that field experiments to elucidate the above problem could be done more easily on these animals than on our first site. Accordingly we proceeded to observe this population and found that they did exhibit a less spectacular rhythm correlated with the day and night periods. During the day it would be possible to find two or three animals lying in the deepest and darkest parts of the cave while at night there were six or seven animals actively moving about close to or actually in the cave entrance. The period of emergence from the back of the cave was about one hour in advance of that on our first open site.

FIGURE 3


holothurian site
rock
15
10
5
0
numbers of holothuria tubulosa
27th
28th
30th-31st July
4th-5th Aug.
1800
2100
2400
0300
0500

Unfortunately this last discovery marked the end of our stay in Malta and we were unable to carry out the field experiments we had planned on this cave population. It would have been possible to block off the entrance to the cave excluding all the external light, and, perhaps, to determine whether absolute light intensities have a controlling effect upon the rhythm. Approaching the problem from another angle, we asked C.C.Hemmings to measure the change in light intensity on our original site. It is interesting that the period of greatest change in light intensity preceded the period of emergence by about half-an-hour.

Lest anyone think this is too clear-cut an answer, we must mention, in closing, the population of Holothuria polii living in Paradise Bay at the north end of the island. Though our work was done at the 'Wied', we did visit Paradise Bay where to our surprise we found fairly large numbers of Holothuria polii (smaller and dark brown) actively moving and apparently feeding on a sandy gravel bottom at midday. A twelve hour watch on these creatures did indicate that they were, in fact, more active and move further at night, but the fact remains that they do not hide beneath the rocks during the day. On the other hand, while going out to map the movements of this sand-living species we noticed that Holothuria tubulosa was emerging from holes in the substrate at the dusk period as we had come to expect.

It must be noted that not only is this sand living creature a different species, but that it does invariably have a covering of sand and gravel on the dorsal surface. This covering of sand may protect the animal from any harmful or noxious effects of the sun and may explain why it does not seem to be as sensitive to white light as Holothuria tubulosa demonstrably is or this may be a camouflage mechanism. Once again it was not possible to pursue this line of investigation any further in the limited time at our disposal. It is clear that we cannot make any generalised statement concerning the behaviour of Holothurians, and therefore have confined ourselves to the observed occurrence of nocturnal behaviour in 'port-holed' and 'non-portholed' varieties of Holothuria tubulosa.

Some Observations on the Diurnal Activity of Fish.

The mobility and intelligence of fish makes catching difficult, and identification is therefore often more difficult still. Nonetheless many species of fish can, with practice, be identified on sight. Thirty seven different species were identified in Wied iz Zurrieq, as well as five other genera of fish which we were unable to identify below genus level. Of these thirty seven species, twenty nine were sufficiently abundant for conclusions to be drawn as to diurnal changes in their behaviour, and of these twenty nine species, six were more abundant by night, thirteen were more abundant by day, and ten were approximately equally abundant day and night. A few examples will be briefly discussed.

More abundant by Night :

Apogon imberbis, a small bright red fish, spends the day in caves or large holes, sometimes in considerable numbers, emerging progressively further from these holes as dusk falls. During the hours of darkness they swim freely, although keeping always fairly close to the substratum.

The Maenidae, recognisable by a characteristic black rectangular area in the middle of the side of the body, appear to spend the day out at sea or fairly near the bottom. At nightfall they swim in towards the coast in shoals which then break down, many individual fish being seen, often lying motionless on the substratum.

Scorpaena sp. Three species of Scorpionfish were seen; all live at the entrance of holes or crannies by day, but emerge at night, lying out on the open bottom or often on top of the Poseidonia. At night the body is often transversely divided by two or three bands of darker red or brown colour. We believe that the curious croaking noises heard only at night originate from these Scorpionfish. Dotu (Kyushu Imp. Univ., Dept. Agri. Bull. Sci. 13, 1951) has described a sound-producing mechanism in the swim-bladder of a Scorpaenid. The behaviour of these fish at night suggests a possible territorial function of these noises.

More abundant by Day :

Chromis chromis is the most abundant of the daytime fishes, keeping close to the coast or to the bottom. At night they are to be found only and very abundantly in holes and crannies in the rock. At dusk they appear to swim closer to the surface (possibly following a particular level of light intensity), then to disperse over the substratum and to select holes by peering in and then backing in slowly. The brilliant blue young, which first appeared in mid-July and which trebled in size within a month, behave in a similar way. At first light Chromis reappear at the entrances, gradually coming out into the open, and forming within half an hour small shoals, and then larger ones. Some colour change was observed, from jet black in courting males to a fairly pale grey in the individuals near the surface at dawn and dusk. Broad pale transverse bands were noted on a few individuals at night, apparently on those not properly in holes.

The Labridae (Wrasses) are virtually never seen at night at all, a marked contrast with their abundance and diversity by day.

Coris julis is found in two different colour forms - the smaller, duller form which is more commonly female, and the larger, brighter form which is more commonly male. The proportion of the latter form increased considerably during July and August, and was significantly more abundant at 85 feet than at 35 feet ($x^2 < 0.1\%$), possibly due only to differences in habitat. Scanty observations in aquaria have given rise to the textbook statement that Coris bury themselves in the sand at night, but it appears probable that they hide in rock crevices. None was ever found at night despite the raking and searching of considerable areas of sand.

Thalossoma pavo too are reputed to bury themselves in the sand at night. At sunset they show shoaling behaviour and congregation near the surface, following one another and occasionally bending the body into L-shapes - possibly intention movements of backing into holes. They then disappear amazingly suddenly, at the time when the light conditions for observation are most difficult. Many painstaking hours of trying to follow these fish at sunset led to only one individual being found at night - an adult male wedged in the same small rock hole each night.

Equally Abundant Day and Night :

Mullus surmuletus were seen to be ubiquitous at all times. By Red Mullet counts, recording numbers, activity, and patterning at different times, we showed that at night these fish are significantly ($x^2 < 0.1\%$) less active and less aggregated than by day; also that at night they lose their longitudinal black and yellow lines and replace them by two or three broad red-brown transverse bands.

Serranus scriba. We carried out counts on Serranus scriba and showed that it is more commonly motionless, and singly on the substratus, and paler at night than by day (x^2 all $< 1\%$). In the similar but scarce Serranus cabrilla, the daytime longitudinal line disappears at night except where it in places helps to form the many dark transverse bands which replace it.

From our observations it appears that those daytime fish with longitudinal markings either hide themselves at night, as exemplified by Coris julis, or else suppress these markings with transverse ones, as shown in Mullus surmuletus and Serranus cabrilla (and in the Festive Cichlid in an aquarium at BCRB's home). Speculation on the reasons for this should perhaps await better knowledge of underwater light conditions by day and night; possibly it is a result simply of reduced activity at night.

Here perhaps one should mention some of the problems in studying diurnal fish behaviour in the wild. Firstly, it is virtually impossible to compare colours due to the differences in lighting conditions when using torches at night, or daylight at different depths. One has therefore to concentrate more on changes of pattern than on changes of colour, although fish can vary their patterns of marking with considerable speed, and diversity, and did so at every possible occasion in response to our torch beams. Secondly, the necessary use of torches at night has considerable effects on the behaviour of nocturnal fishes - Scorpaena, Maena, and Mullus appear to be mesmorised; most other fish avoid the torch-beam with varying degrees of haste. The use of red torches was ineffective. Thirdly, there are effects on the observer himself, notably the very great reduction in his visual field, of the use of torches which can only illuminate a limited area.

General Observations on some other Invertebrates.

Observations to provide a general background to our specialised studies indicate that diurnal activities occur in almost all of the major invertebrate phyla found in the Wied, and these observations do indicate that there is here a vaste field of research awaiting detailed study.

Many Crustacea are more active at night. The large hermit crabs with their cargo of commensual anemones are often encountered on a night dive yet hardly ever seen in daylight. Counts of the number of minute hermit crabs, found on the rock scree, in a given area showed these to be twice as numerous at night, and they fall in showers from the rocks wherever torches are shone on them. The Mysids, however, spend the day constantly swimming round in stationary shoals, consisting of individuals of the same size. These shoals break up at night and it would be interesting to determine how they are formed again next day.

Caves and hollows are transformed at night by the white and red, tentacles of sessile, tube-dwelling Serpulid worms, which are completely retracted during the day.

A number of counts at night, coupled with flash photography indicate that certain Ophiuroids crawl out from crannies at dusk and spend some of the night moving around over the substrate possibly searching for food.

General.

The team was housed in a large ground floor flat in Sliema on the north coast some five miles from the 'Wied'. Transport was by a hired Commer Diesel van which had ample room for eight people and all diving equipment. Two air supplies were available, one at the Naval Diving Unit Manoel Island, and the other, as reserve, at RAF Luqa. Our equipment consisted of commercial demand valves and aqualungs, and a mountain of other gear. We were unlucky to have many equipment faults, on which much time and trouble was spent.

As a result of the heat, and our somewhat erratic hours due to the diving programme, some of us felt considerably under-the-weather at times, and some useful diving was lost. However, in just over five weeks diving, 210 dives, totalling 158 hours underwater were carried out. At times care had to be taken to observe the multiple-dive Decompression Tables, and decompression of up to 25 minutes was done after routine work dives on occasions. The argument of small versus large diving teams has often been raised, but it is certain that we should have benefitted from a larger team.

A number of general lessons were learned this summer. The necessity for an advance party to make all domestic arrangements - housing, vehicle, customs clearance for equipment, etc - is clear. In addition it is essential to have one member with first-hand knowledge of the potential diving sites, especially where they are relevant to the intended project - exploratory diving wasted a further five days, so that our actual experimental work was restricted to less than five weeks.

Concerning diving technique, the most interesting problem to come out of our work is that of accurate observation of marine life at night. Mention of the problems has already been made, and as the dark-adapted eye is not sufficient artificial aids must be used. Much experimentation with lighting techniques, the use of flash photography, and more sophisticated equipment - semi-rebreathing mixture sets, underwater houses - is needed. We would venture to suggest that continuous watches of the sort described here, and more protracted observation - of 48, 72 or more hours - can only be done properly from an underwater base, however good the diving team.

Finally a special note of thanks to the two girls on the trip - Anne and Sue - who had much to put up with, and looked after us superbly.

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Collins and Chambers Ltd.

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Sub-Aqua Products, Eastleigh, Ltd.

Fenn Aqua Sport Ltd.

Tillbrook & Co.Ltd.

Phillips Electrical Ltd.

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Income

Grants:	Worts Fund	£ 50.	0.	0.
	Sembal Trust	£100.	0.	0.
	Shell Co.	£ 50.	0.	0.
	Challanger	£ 25.	0.	0.
	Mosley	£100.	0.	0.
	Trinity Hall	£ 50.	0.	0.
	Clare	£ 40.	0.	0.
	St. Catharine's	£ 20.	0.	0.
	CUUEG	£ 20.	0.	0.
		£455.	0.	0.
Personal Contributions:		£446.	12.	9.
Other Payments:		£ 9.	13.	0.
Total Income:		£911.	5.	9.

Expenditure

Equipment:	£221.	7.	0.
Living Expenses:	£198.	10.	0.
Air Flights:	£282.	19.	0.
Insurance:	£ 65.	6.	1.
Vehicle Expenses:	£ 91.	1.	8.
Postage, Stationery, etc.:	£ 13.	13.	9.
Debts:	£ 6.	14.	9.
	£879.	12.	3.
Credit in Bank for Report & Distribution	£ 31.	13.	6.
Total Expenditure:	£911.	5.	9.